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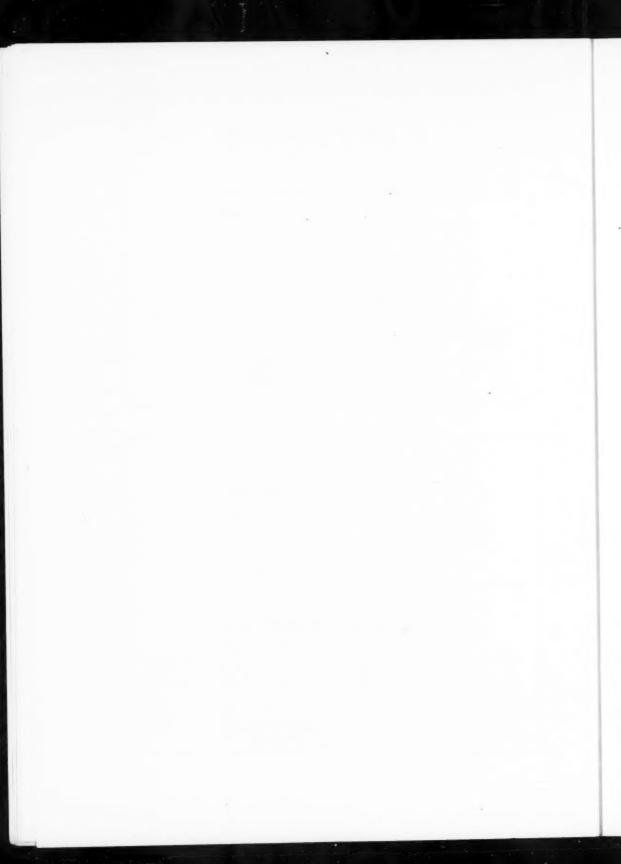
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ANALYTICAL APPLICATION OF THE SEPARATION OF IRON-III FROM ALUMINIUM EMPLOYING ANIONIC EXCHANGE RESIN

by

L. D. C. BOK and V. C. O. SCHULER

OPSOMMING

As 'n oplossing van yster-III en aluminium in gekonsentreerde soutsuur deurgestuur word deur 'n kolom van die anioon-uitruilingsharpuis Amberlite IRA 400, word die yster bevredigend teruggehou, en die aluminium gaan deur. Die yster kan uitgewas word met N soutsuur. Die invloed van titanium word beskou. Toepassings word gegee vir die bepaling van yster en aluminium in sement en in ammonium-diuranaat.

SUMMARY

When a solution of iron-III and aluminium in concentrated hydrochloric acid is passed through a column of the anion exchange resin Amberlite IRA 400, the iron is satisfactorily retained, and the aluminium passes through. The iron can be eluted with N hydrochloric acid. The effect of titanium is considered. Applications are given for the determination of iron and aluminium in cement and in ammonium diuranate.

The adsorption of iron-III from hydrochloric acid solution has been reported by Kraus and Moore[1]. Adsorption was stated to be probably caused by the formation of a negatively charged $[FeCl_4]^-$ complex. Reents and Kahler[2] have reported an industrial application of this in the removal of iron from hydrochloric acid.

In the investigation described below, an attempt has been made to separate iron-III from aluminium by passing the hydrochloric acid solution through an anion exchange column. An advantage this procedure has over the ferric thiocyanate complex method of separation of Teicher and Gordon[3] is that the resin columns can be rapidly regenerated, and the iron can be determined in the effluent. A 30 ml. resin column will retain virtually no iron after elution with 250 ml. N hydrochloric acid. This is probably because on elution the iron which has been adsorbed from concentrated hydrochloric acid as an anionic complex $[FeCl_4]^-$ is converted to cationic Fe^{+++} and the eluting process becomes a washing stage rather than a displacement process. It was found that it required at least 1 litre of either 3N or 4N hydrochloric acid to elute 2 mg. iron-III as thiocyanate from a similar anion exchange resin column.

EXPERIMENTAL

Materials used: Resin: Rohm & Haas Company—Amberlite I.R.A. 400 (Strongly basic anion exchanger). *Hydrochloric acid:* A.R.Q. (A.E. and C.I.) *Iron:* Merck's pure iron wire. *Aluminium:* B.D.H. Reagent Grade aluminium turnings.

Exchange columns: The columns employed were of the Jones Reductor type with the following approximate dimensions: total length above sintered glass filter disc 28 cm.; internal diameter 1·5 cm. except the top 5 cm. which was flared to 2·5 cm. internal diameter. A mark at 30 ml. capacity was made by pouring in this volume of water and marking the level.

Treatment of resin: The resin, as received, was first soaked in approximately N hydrochloric acid for several days. Suitable quantities of the resin (30 ml.) were then transferred as a slurry to the exchange columns. The resin in the columns was then eluted alternately with 100 ml. N hydrochloric acid and 100 ml. concentrated hydrochloric acid.

Procedure for column runs: Before any sorption step the columns were flushed with several bed volumes of the solvent which was to be used in the actual determination. A final check for iron was made on the effluent by the thiocyanate test, which according to Vogel[4], has a sensitivity of $0 \cdot 25 \mu g$. Fe^{+++}, when applied as a spot test. The level of the liquid was never allowed to fall below the top of the resin bed, successive additions being made to the columns just as the last of the remaining solution entered the resin. A flow rate of approx. 2 ml. per minute was maintained unless otherwise stated.

Analytical methods employed: Iron was determined by the colorimetric thiocynate method[5]. For *aluminium* the phosphate precipitation method[6] was used, as it was found that precipitation of the hydroxide with ammonia and subsequent ignition to Al_2O_3 gave high results unless the precipitates were ignited to 1200°. The normality of the *concentrated hydrochloric acid* used was determined with anhydrous sodium carbonate[7] and found to be 10·35.

RESULTS

Efficiency of iron-III adsorption from concentrated hydrochloric acid solution: Aliquots of a standard iron solution were taken to dryness without baking with 0·5 ml. concentrated nitric acid to ensure that the iron was in the III-form. Reents and Kahler[2] state that iron-II does not form the chloride complex. After cooling, the residues were dissolved in 25 ml. concentrated hydrochloric acid. Where dissolution was difficult, the solutions were warmed to 40-50° for a short while. These solutions were then passed through the resin columns previously flushed with concentrated hydrochloric acid. The columns were then washed with ten 10 ml. portions of concentrated hydrochloric acid.

The effluents and washings were combined and evaporated to approximately 10 ml., 0·5 ml. concentrated nitric acid was added, and the solutions taken to dryness. The residues were taken up in 5 ml. 3N hydrochloric acid and the iron determined by the thiocyanate method. The results are given in Table I.

TABLE I

Efficiency of iron-III adsorption from concentrated hydrochloric acid solution.

Fe taken (mg.)	Fe in Effluent (mg.)	% Adsorption
5·0	0·012	99·8
5·0	0·010	99·8
7·5	0·016	99·8
7·5	0·020	99·7
10·0	0·035	99·7
10·0	0·034	99·7
12·5	0·050	99·6
12·5	0·021	99·7

Thus it can be seen that in the range of the concentrations tested, only about 0·01-0·04 mg. iron will be found in the effluent. If aluminium is determined in the effluent contamination by iron will be insignificant.

Effect of acid concentration on adsorption: Aliquots containing 5 mg. iron were taken to dryness with 0·5 ml. concentrated nitric acid as above and taken up in 25 ml. hydrochloric acid at various dilutions. These solutions were then passed through resin columns which had previously been washed with ten 10 ml. portions of hydrochloric acid of corresponding concentration. The iron in the effluents was then determined. It was found (see Table II) that very little leakage of iron occurred even when the acid concentration was reduced to 1 : 1 (approximately 5N). At a normality of about 4N, however, appreciable leakage was observed.

TABLE II

Effect of acid concentration on adsorption
5·0 mg. iron taken.

Acid concentration	Fe in effluent (mg.)	% Adsorption
10·4N	0·012	99·8
10·4N	0·010	99·8
9·4N	0·022	99·6
9·4N	0·017	99·7
8·3N	0·021	99·6
8·3N	0·018	99·6
6·7N	0·022	99·6
6·7N	0·025	99·5
6·2N	0·019	99·6
6·2N	0·018	99·6
5·2N	0·030	99·4
5·2N	0·018	99·6
4·2N	0·35	93·0
4·2N	0·52	89·6

Elution of iron from columns: Even when the acid strength is reduced to 5N hydrochloric acid the $[FeCl_4]^-$ ion can be effectively adsorbed but at lower strengths significant leakage occurs, although Kraus and Moore [1] report adsorption of $[FeCl_4]^-$ from 3N hydrochloric acid solution. Reents and Kahler [2] describe industrial methods for the removal of iron from hydrochloric acid, with subsequent removal of iron from the columns by flushing with water.

As iron-III precipitates out [8] at a pH 2-3, it was thought preferable to use approx. N hydrochloric acid to remove the iron from the columns.

The columns used for determining the efficiency of adsorption from concentrated hydrochloric acid were eluted with N hydrochloric acid and the eluates were collected in 250 ml. graduated flasks. Elution was conducted at the same flow rate as the initial sorption. Enough elutriant was passed through the columns to make the volume up to 250 ml. in the collecting flasks. The iron in the eluates was then determined. The results in Table III indicate that 250 ml. N hydrochloric acid will remove practically all the iron from a 30 ml. resin column.

TABLE III

Elution with N hydrochloric acid.

Fe taken (mg.)	Fe found (mg.)
5·0	5·0
5·0	5·0
7·5	7·4
7·5	7·4
10·0	10·0
10·0	10·0
12·5	12·4
12·5	12·4

Separation of mixtures of aluminium and iron-III: To determine whether iron and aluminium can be satisfactorily separated in hydrochloric acid solution by means of ion exchange, mixtures containing 5 mg. iron and from 10 to 50 mg. aluminium in 25 ml. concentrated hydrochloric acid were prepared. After passage through the resin columns and washing with concentrated hydrochloric acid, the effluents were evaporated to approximately 10 ml. and the aluminium determined by the phosphate method. The iron was removed from the columns by elution with 250 ml. N hydrochloric acid and then determined by the thiocyanate method. The results in Table IV indicate that a good separation of these metals can be achieved.

TABLE IV

Separation of iron-III from aluminium.

Fe taken (mg.)	Al taken (mg.)	Fe found (mg.)	Al found (mg.)
5·0	10·0	5·0	10·0
5·0	10·0	4·9	9·9
5·0	20·0	5·0	20·3
5·0	20·0	5·0	20·3
5·0	20·0	5·0	20·3
5·0	50·0	5·0	50·4
5·0	50·0	5·0	49·7

Effect of titanium: As titanium is often associated with iron and aluminium in ores, its behaviour when passed through an anion exchange column in concentrated hydrochloric acid solution was investigated.

A solution of 5 mg. titanium in 25 ml. concentrated hydrochloric acid was passed through an anion exchange column. After some 25 ml. had entered the resin bed, a positive test for titanium was obtained in the effluent by means of the hydrogen peroxide test.

It was considered that the yellow complex of titanium with hydrogen peroxide might possibly be retained on the resin. Accordingly, 100 volume hydrogen peroxide was added to 5 mg. titanium in 25 ml. concentrated hydrochloric acid until no further

darkening of the solution occurred. A column of IRA 400 resin was flushed with hydrochloric acid containing hydrogen peroxide and the yellow peroxidised titanium solution was then passed through. The effluent had practically the same colour as the original solution, indicating that the complex is not appreciably adsorbed.

To test whether any titanium in concentrated hydrochloric acid solution is retained on the resin, known amounts were passed through the columns, followed by ten 10 ml. washes of concentrated hydrochloric acid. The effluents were made up to 250 ml., suitable aliquots removed, and the titanium determined by the peroxide method[9]. From the results in table V it appears that the columns retain some 10% of the titanium after a 100 ml. wash. The columns were then washed with a further 25 ml. concentrated hydrochloric acid and the titanium determined in the effluents. These too contained titanium, but complete recovery of the titanium was not effected even after an additional 25 ml. wash. The results show that to collect all the titanium in the effluent a wash of more than 100 ml. would be necessary, a quantity sufficient to wash through at least 50 mg. of aluminium.

TABLE V

Recovery of titanium with concentrated hydrochloric acid.

Ti taken (mg.)	Ti in 1st wash with 100 ml. (mg.)	Ti in 2nd wash with an extra 25 ml. (mg.)	Ti in 3rd wash with a further 25 ml. (mg.)
1·0	0·9		
1·0	0·9		
5·0	4·5		
5·0	4·4		
10·0	8·5	0·96	0·48
10·0	8·5	0·88	0·44

Interference of titanium in aluminium determinations: Most of the conventional methods for the determination of aluminium require the absence of titanium, viz:—(i) Precipitation with ammonia as aluminium hydroxide—titanium will also hydrolyse and precipitate. (ii) 8-Hydroxyquinoline—according to Flagg[10] the respective aluminium and titanium "oxinates" precipitate completely in the following pH-ranges: Aluminium 4·2-9·8; Titanium 4·8-8·6. This indicates that a separation of the two metals is not easily achieved by oxine. (iii) Phosphate method—Hillebrand, Lundell, Bright and Hoffman[11] state that titanium interferes.

It is thus essential to remove any titanium before attempting to determine aluminium after the iron has been separated by the ion exchange procedure.

It is stated that small amounts of titanium can be separated from proportionately large amounts of aluminium by precipitating the former with 6% aqueous cupferron in 3N hydrochloric acid solution[12]. This procedure was tested by preparing solutions containing known quantities of aluminium and titanium. The titanium was precipitated with cupferron as above, the titanium cupferrate filtered off and washed well with dilute hydrochloric acid. The filtrate containing the aluminium was evaporated to small volume, and the excess cupferron destroyed by fuming off with sulphuric and nitric acids. The aluminium was then determined by the phosphate method. Solutions containing like amounts of aluminium but no titanium were also analysed for comparison. The results are given in Table VI.

TABLE VI

Interference of titanium in aluminium determinations.

Al taken (mg.)	Ti taken (mg.) and afterwards separated with cupferron	Al found (mg.)
20·0	—	20·3
20·0	—	20·5
20·0	2·0	20·4
20·0	2·0	20·4

The separation of titanium from aluminium by differential hydrolysis in acetic acid—acetate buffer [13] was also investigated, but it was found that the hydrolysed titanium from dilute solution gave a fine suspension which proved very difficult to filter.

The determination of aluminium in materials such as bauxite, containing titanium, may thus be accomplished by first removing the iron by an exchange process, separating the titanium with cupferron and finally precipitating aluminium.

Practical applications.

(a) *Determination of alumina in cement.*

0·25G. of a commercially available standard analysed sample was moistened with 10 ml. water, 5 ml. concentrated hydrochloric acid were added, and the suspension was digested and agitated with a glass rod until solution was complete. The solution was then taken to dryness to dehydrate the silica. The residue was treated with 5 ml. concentrated hydrochloric acid, diluted to 20 ml., and heated to dissolve the salts. It was then filtered immediately and washed with dilute hydrochloric acid. The filtrate was heated to boiling and neutralised to methyl red with ammonia, boiled for one minute, filtered and washed with 5% ammonium nitrate solution. The precipitate was redissolved in hydrochloric acid and the above precipitation and filtration repeated to separate calcium and magnesium. The hydroxide precipitate was then dissolved in a minimum of hydrochloric acid and evaporated to dryness. The residue was dissolved in 25 ml. concentrated hydrochloric acid and passed through a 30 ml. Aberlite IRA 400 resin column previously flushed with concentrated hydrochloric acid. The column was then washed with ten 10 ml. portions of concentrated hydrochloric acid.

As the sample contained no titanium, the effluent was evaporated to 25 ml., and the aluminium determined by the phosphate method. The result was in good agreement with the value guaranteed by the suppliers.

(b) *Determination of alumina in ammonium diuranate "cake".*

2·0G. of the dry sample was dissolved in a minimum of nitric acid, 10 ml. concentrated hydrochloric acid added, the solution evaporated to dryness and baked for 1 hour at 110° to dehydrate any silica. The residue was treated with 100 ml. 10% hydrochloric acid and heated to dissolve the salts. The silica was filtered off on a Whatman No. 42 filter paper and washed with 5% hot hydrochloric acid. The filtrate was heated to near boiling and ammonia added until the first permanent precipitate appeared. Ammonium carbonate and more ammonia were added until the solution was just alkaline to litmus, after which the solution was boiled for one

minute and filtered. (The uranium compound is soluble in alkaline carbonate solution.) After washing with 5% ammonium carbonate solution, the precipitate of iron and aluminium was dissolved in hydrochloric acid and the above separation repeated to ensure the complete separation of uranium.

The iron and aluminium hydroxides were then dissolved in hydrochloric acid and evaporated to dryness with a little nitric acid. The residue was taken up in 25 ml. concentrated hydrochloric acid. The iron was then separated by passing the solution through a 30 ml. IRA 400 resin column and washing with concentrated hydrochloric acid. The effluent was evaporated to about 20 ml. and then diluted to 300 ml. The solution was tested for the presence of titanium by adding a few drops of hydrogen peroxide (100 vol.). No yellow colour was formed, indicating the absence of titanium. The aluminium was then determined by the phosphate method.

As no standard analysed sample of this material was available to us, known amounts of aluminium were added to 2·0 g. ammonium diuranate samples and taken through the whole process. Table VII shows that the alumina recovered is comparable with the additions made.

TABLE VII

2·0G. *ammonium diuranate with alumina additions.*

Al_2O_3 added (mg.)	Total Al_2O_3 found (mg.)	Difference	% Al_2O_3 in original sample
Nil	19·4		
		19·4	0·97
Nil	19·4		
10	30·4		
		19·8	0·99
10	29·1		
20	38·6		
		19·6	0·98
20	40·6		
40	59·0		
		19·2	0·96
40	59·3		

The iron from the columns was eluted with N hydrochloric acid and the eluates made up to 250 ml. The iron in these solutions was then determined colorimetrically by the thiocyanate method. The results varied from 0·28% to 0·30% iron.

A method for determining both iron and aluminium on the same sample is thus available.

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THE ACCURACY OF pH DETERMINATIONS WITH A GLASS ELECTRODE

by

D. A. CLUR and N. P. FINKELSTEIN

OPSOMMING

Die fout van die vloeistof kontakpotensiaal in pH bepaalings is met behulp van 'n pH meter onder praktiese omstandighede ondersoek. Verskeie maniere om vloeistof kontakte te verkry is vergelyk, en 'n metode word beskryf wat die reproduseerbaarheid van die fout tot binne 0·01 van 'n pH eenheid verseker.

SUMMARY

The liquid junction potential error in pH measurements with a glass electrode has been studied under practical conditions using a pH meter. Various methods of making liquid junctions have been compared, and a procedure is given which ensures a reproducibility of the error to within 0·01 pH units.

Certain high precision solubility measurements at present being undertaken in this Department necessitate a knowledge of the pH's of unbuffered solutions accurate to at least 0·01 pH unit. Although pH's are generally quoted and regarded accurate to 0·01-0·02 pH units, theoretical considerations indicate that residual liquid junction potentials involved must give rise to errors far in excess of this limit. Dole[1] mentions that these may be as great as 0·1 pH unit, but does not appear to have adequate experimental support for this statement. It was therefore essential that these conflicting views should be resolved before the accurate determinations envisaged could be carried out. Accordingly, it is the purpose of this investigation to demonstrate the order of magnitude of the error due to liquid junction potentials under the conditions pertaining during pH measurements, and in addition to show the significance of the common neglect of certain fundamental properties of liquid junctions.

It has been shown[2] that the magnitude, stability and reproducibility of liquid junctions depend, among other things, on the way in which the liquid junction is made. A satisfactory junction should give a stable, reproducible potential, and in addition, when used with pH meters, should be simple to set up. The junctions usually embodied in the electrodes of commercial meters are chosen primarily to satisfy the requirements of convenience, for they are what Guggenheim[2] calls "indefinite type" junctions. These he found to be extremely irreproducible and unstable. He also showed that in order to satisfy the requirements of stability a junction must be made so as to have "cylindrical symmetry," i.e. all concentration and potential gradients must be parallel to the axis of the tube. This is achieved, in practice, by forming the junction in a tube of regular bore, at a point some distance from its ends. Normally this type of junction is not used with pH meters, in spite of its advantages and the fact that, as will be shown, it can be very simply set up.

EXPERIMENTAL

The cell studied was a conventional pH cell:

Ag-AgCl	HCl	glass	Potassium hydrogen phthalate pH=4·00	Satd. KCl	Hg₂Cl₂-Hg

The liquid junction between the buffer and saturated potassium chloride solution was prepared in different ways, and the variation of pH with time observed for each type of junction. Any change in the measured pH can only be caused by variations in the boundary layer, and hence in the junction potential. Care was taken to follow, as far as possible, the procedures usually used in pH work. Thus, the meter was set on the standard value of 4·00 pH units immediately after the junction had been made.

The pH meter used was a Marconi Type TF 717A, which embodies a potentiometric measuring circuit. A Sorensen Model 1001 voltage regulator, and a constant voltage transformer were used to eliminate the effects of mains fluctuations. In addition, to ensure that any pH fluctuation was due to unsteady junction potentials, the stability and reproducibility of the meter were checked by balancing it against a standard potential. No change in meter reading was observed over 16 hours. As the meter used has automatic temperature compensation, temperature effects could be neglected.

Four types of liquid junction were considered:

(a) Wick junction (as in the Beckmann electrode).

(b) Porous plug junction (as in the Marconi electrode).

(c) Simple dipping junction. This is one widely used with common laboratory calomel electrodes. The two solutions make contact at the end of a tube, filled with the first, and dipping into the second. In Type A the saturated potassium chloride solution was the upper, and in Type B the lower of the two solutions.

(d) Cylindrically symmetrical junction. This was made as follows: The path DBCE (see Fig. 1) of a 120° three-way tap was completely filled with buffer solution, and any solution projecting beyond D and E removed with a piece of filter paper. With the tap turned so as to seal all three limbs, this bridge was carefully lowered, until D and E dipped into the buffer and saturated potassium chloride solution containers respectively. A and B were connected, and potassium chloride solution slowly drawn about two cms. up DB. Again all the limbs were sealed, and a tight fitting rubber bung inserted in F. The tap was now turned to connect A and C momentarily, and then B and C. By this means it was ensured that the sharp boundary in DB was not disturbed when B and C were connected. The saturated potassium chloride and buffer solutions were now in contact at a point in DB. This junction may be used with commercial electrodes, as shown in Fig. 1. No junction potential arises at the plug or wick of the commercial electrode as it is dipping into a saturated potassium chloride solution.

RESULTS

In Table 1 is given an analysis of the readings taken with the various types of junction. These are considered under three headings, viz. the readings taken during the first two hours after the junction had been set up, the readings taken after this period, and the readings taken during the whole period of the run. An indication of the stability of the readings in each of the cases treated is given by the mean deviation from the mean of the readings during each of the three periods, while the mean

pH during any period from the pH at which the meter was originally set indicates the reproducibility of the liquid junction potential. The data in Table 1 are summarised in Table 2 to facilitate comparison, and in addition, two other important stability characteristics are shown: the time which elapsed between the establishment of the junction and the attainment of a period of stability, and the duration of the period of stability.
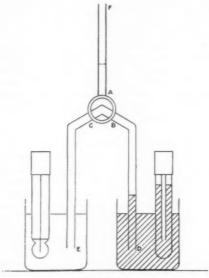
Fig. 1

Before considering the implications of these data, the meaning of the term "period of stability" should be clarified. It was found that in all cases except that of the simple dipping junction Type A the making of the junction was followed by wide fluctuations of potential, which gradually decreased in magnitude until a steady state was reached. In this steady state variations of potential still occurred, but their magnitude remained constant. It is the period during which this steady state persisted which is known as the "stable period". Both the time taken for the stable period to be arrived at, and its duration, varied from run to run, but the limits within which the steady state pH varied were less random, and clearly depended on the type of junction concerned. The figures in the Tables show that the porous plug, and simple dipping Type A junctions were the least stable. The potential at the latter, indeed, varied randomly during the whole period of a run, and no steady state could ever be detected. While the overall stability of the simple dipping Type B (±0.017 pH unit), wick (±0.013 pH unit,) and cylindrically symmetrical junctions (±0.016 pH unit) were very similar, it is evident that the stability of the last was considerably greater than that of any other type (±0.002 0H unit) once the steady state was reached.

Two other marked differences in the behaviour of cylindrically symmetrical and conventional junctions are noteworthy. The first of these is the fact that the increase in stability, as the junctions approach the steady state was much greater for

cylindrically symmetrical junctions; the second is that the steady rate did not deteriorate in the case of cylindrically symmetrical junctions. Readings were taken for as long as 24 hours without any decrease in stability becoming manifest. In the other cases, however, it was found that the magnitude of the variations in pH increased after the steady state had been in existence for some hours.

A striking feature of the results is the degree of irreproducibility of the potentials at all the types of junction treated above. They indicate that the most reproducible potentials are those at wick type junctions, and that these are considerably more satisfactory than the potentials at cylindrically symmetrical junctions. Even with wick junctions, however, it must be expected that irreproducibility at the liquid junction will introduce an uncertainty of 0·040 pH units in any complete determination. If in addition, the instability of the junction is considered, the resultant uncertainty is seen to be of the order of 0·060 pH units. The significance of this figure is apparent if it is considered in the light of the statement made at the beginning of this paper—that pH's measured by the techniques described above are commonly regarded as being accurate to within 0·01 to 0·02 pH units.

Further considerations of cylindrically symetrical junctions

It was considered that the irreproducibility shown above by cylindrically symmetrical junctions was not inherent in the junctions themselves, but was caused by a fundamental error in the conventional pH determination procedure used here, which involves setting the meter, or reading the pH, immediately after the junction is made. The above results, however, as well as theory,[3] and accurate experimental work[4], [5] show that junction potentials, and in particular those at cylindrically symmetrical junctions, are unstable directly after their formation, but gradually steady with time. Thus, the conventional procedure leads to readings being taken, and the meter being standardised, at the most unfavourable time. It would surely be more satisfactory if the initial instabilities were allowed to die away, and the steady state reached, before reading or setting the meter.

To prove this theory, another set of readings was made with cylindrically symmetrical junctions using the following procedure. A cylindrically symmetrical junction was set up as described above, and after a lapse of two hours the meter was set at the pH of the buffer. A new junction was then made and its pH followed until the steady state was reached. The results are shown in Table 3a. They show that this modification indeed produces a marked improvement, and reduces the uncertainty in a complete pH determination to within 0·01 pH units.

The very real drawback of this method is that 2 to 3 hours are necessary for each pH reading. It was, however, also found possible to reduce considerably the time taken to set up the steady state by taking special precautions to minimise mixing of the two solutions as the junction is made. These consisted of siliconing the walls of the tube and carefully grinding flat its end. The marked improvement resulting is shown in Table 3b. Initial fluctuations were smaller and stability was reached in less than 30 minutes. Thus, it is possible to reduce the time necessary for a reading to half an hour without sacrifice in accuracy.

It must be emphasised, however, that even though stable reproducible junction potentials can be obtained by this careful technique, the actual junction potential error is not thereby eliminated. To minimise this it is necessary, in addition, to ensure that the pH of the buffer used for standardising the meter be as near as possible to that of the unknown solution, in which case the two junction potentials will tend to cancel.

TABLE 1

Behaviour of various junction types studied

Porous plug junction / *Simple dipping junction*

Experiment number	1	2	3	4	5	6	7	Mean	1	2	3	Mean
Mean deviation from mean for results taken:												
(1) during the first two hours	0·031	0·007	0·007	0·041	0·033	0·014	0·051	0·026	0·018	0·015	0·017	0·017
(2) after the first two hours	0·018	0·011	0·009	0·010	0·055	—	0·019	0·020	0·012	0·017	0·007	0·012
(3) for the complete run	0·093	0·008	0·009	0·067	0·056	0·015	0·036	0·040	0·016	0·018	0·016	0·017
Difference between pH meter and mean value obtained:												
(1) during the first two hours	−0·012	+0·013	+0·011	−0·039	+0·026	−0·028	+0·017	−0·010	−0·022	−0·025	−0·043	−0·030
(2) after the first two hours	−0·110	+0·011	+0·013	−0·167	+0·138	—	+0·051	0·070	−0·022	−0·052	−0·059	−0·044
(3) for the complete run	−0·052	+0·006	+0·013	−0·109	+0·067	−0·031	+0·041	−0·046	−0·021	−0·047	−0·053	−0·040
Number of readings:												
(1) during the first two hours	14	10	7	5	7	9	8		5	4	7	
(2) after the first two hours	9	7	13	6	4	1	16		9	20	7	
(3) for the complete run	23	17	20	11	11	10	24		14	24	14	

Wick junction / *Simple dipping junction*

Experiment number	1	2	3	4	5	6	7	Mean	8	9	10	Mean
Mean deviation from mean for results taken:												
(1) during the first two hours	0·004	0·014	0·012	0·010	0·013	0·008	0·014	0·010	0·010	0·010	0·006	0·010
(2) after the first two hours	0·009	0·006	0·024	0·007	0·007	0·009	0·006	0·006	0·008	0·008	0·021	0·010
(3) for the complete run	0·014	0·011	0·021	0·009	0·013	0·009	0·013	0·013	0·015	0·009	0·018	0·013
Difference between pH meter setting and mean value obtained:												
(1) during the first two hours	−0·007	−0·016	+0·010	−0·008	−0·010	−0·015	−0·022	−0·017	+0·031	+0·005	+0·047	+0·017
(2) after the first two hours	−0·049	0·000	+0·034	−0·016	−0·027	+0·005	−0·032	−0·025	+0·063	+0·003	+0·021	0·025
(3) for the complete run	−0·033	−0·006	+0·023	−0·010	−0·019	−0·007	−0·027	−0·020	+0·041	+0·004	+0·032	0·020
Number of readings:												
(1) during the first two hours	4	7	5	5	7	4	13		15	2	3	
(2) after the first two hours	14	10	6	8	7	14	5		7	5	4	
(3) for the complete run	18	17	11	13	14	18	18		22	7	7	

TABLE 1—*continued*

Behaviour of various junction types studied

Cylindrical Symmetrical junction

Experiment number	1	2	3	4	5	6	7	8	9	10	Mean
Mean deviation from mean for results taken:											
(1) during the first two hours	0·005	0·041	0·003	0·025	0·028	0·012	0·009	0·006	0·013	0·032	0·017
(2) after the first two hours	0·002	0·003	0·003	0·002	0·002	0·002	0·002	0·002	0·004	0·002	0·002
(3) for the complete run	0·004	0·033	0·013	0·026	0·012	0·011	0·007	0·011	0·012	0·027	0·016
Difference between pH meter setting and mean value obtained:											
(1) during the first two hours	+0·013	−0·046	−0·006	−0·044	+0·032	−0·048	−0·013	−0·029	+0·016	+0·015	0·026
(2) after the first two hours	+0·012	−0·111	−0·028	−0·089	+0·046	−0·029	−0·007	−0·006	−0·007	−0·036	0·037
(3) for the complete run	+0·013	−0·088	−0·019	−0·059	+0·039	−0·036	−0·011	−0·018	+0·009	−0·014	0·031
Number of readings:											
(1) during the first two hours	13	6	5	11	3	3	11	10	12	6	
(2) after the first two hours	7	5	7	5	9	4	6	11	6	8	
(3) for the complete run	20	11	12	16	12	7	17	21	18	14	

TABLE 2

Summary of the behaviour of junction types studied

Junction type	Simple Dipping		Wick	Porous Plug	Cylindrically	Symmetrical
	Type A	Type B			Unsiliconed	Siliconed
Mean deviation from mean for readings taken:						
(1) during the first two hours of run	Completely unstable	0·017	0·010	0·026	0·017	0·004
(2) after first two hours of run		0·012	0·010	0·020	0·002	0·001
(3) during the complete run		0·017	0·013	0·040	0·016	0·004
Difference between original meter setting and mean pH.						
(1) during the first two hours of run		0·030	0·017	0·019	0·026	0·005
(2) after first two hours of run		0·044	0·025	0·070	0·037	0·011
(3) during the complete run		0·040	0·020	0·046	0·031	0·008
Time taken to reach stability (hours)		0—3	0—2	0—3	1—2·5	0—0·5
Period of stability in hours		2—7	3— >5	4	>5	>5

TABLE 3

Results with meter set at buffer pH during "steady rate"

Experiment number	A. Unsiliconed				B. Siliconed			
	1	2	3	Mean	1	2	3	Mean
Mean deviation from the mean of readings taken:								
(1) during 1st two hours	0·041	0·006	0·012	0·020	0·002	0·004	0·002	0·003
(2) after 1st two hours	0·003	0·002	0·002	0·002	0·002	0·003	0·000	0·002
(3) during the complete run	0·033	0·011	0·011	0·018	0·006	0·003	0·003	0·004
Difference between original meter setting and mean pH								
(1) during the first two hours	+0·064	−0·022	−0·018	0·035	−0·012	+0·011	−0·002	0·008
(2) after the first two hours	−0·003	+0·002	+0·003	0·003	−0·001	+0·012	+0·005	0·006
(3) during the complete run	+0·022	−0·011	−0·006	0·013	−0·007	+0·011	+0·003	0·007
Number of readings:								
(1) during the first two hours	6	10	5		12	8	7	
(2) after the first two hours	5	11	7		9	11	11	
(3) for the complete run	11	21	12		21	19	18	

CONCLUSIONS

It has been shown that with the equipment generally used in pH determinations an uncertainty of at least 0·1 pH unit must be expected in any measurement, but that this can be reduced to 0·01 pH units by using a free diffusion junction with "cylindrical symmetry".

The results of this purely practical investigation substantiate the statement by Dole[1] that the potentials at indefinite type junctions "may be in error by as much as 3-5 mV or 0·1 pH unit", although, where the junction is cylindrically symmetrical, "fluctuations in its potential would be less than 0·01 pH unit".

ACKNOWLEDGEMENTS

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PREPARATION OF DOUBLE METAL SULPHIDES OF THE TYPE AB₂S₄.
PART III. COMPOUNDS OF MOLYBDENUM AND TANTALUM.

by

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OPSOMMING

Twee dubbelmetaal-sulfiede, $MoBe_2S_4$ en $TaTi_2S_4$, is in reaksies in die vaste toestand by hoë temperatuur berei. Verder is $MoTl_2S_4$ en $MoCu_2S_4$ berei as 'n neerslag uit oplossing. $MoBe_2S_4$ en $MoCu_2S_4$ is spinelle, en die ander is ook kubies.

SUMMARY

Two double metal sulphides, $MoBe_2S_4$ and $TaTi_2S_4$, have been prepared in reactions in the solid state at high temperature. In addition, $MoTl_2S_4$ and $MoCu_2S_4$ have been prepared as precipitates from solution. $MoBe_2S_4$ and $MoCu_2S_4$ are spinels, and the others are also cubic.

EXPERIMENTAL

Three methods were used in attempts at preparing double metal sulphides:—

(1) *Heating of the constituent elements, or binary sulphides*, in stoichiometric proportions in sealed evacuated tubes (Part I, Method (i) [1]).

(2) *Precipitation from aqueous solution.* Precipitates of double metal sulphides were formed when solutions of the salts of certain metals were added to a solution of ammonium sulphomolybdate $(NH_4)_2MoS_4$. This was prepared by adding ammonium hydroxide (50 ml., S.G. 0·94) to a solution of $(NH_4)_6Mo_7O_{24}.4H_2O$ (5g.) in water (15 ml.) and passing hydrogen sulphide through the liquid until blood red tabular crystals with a green shimmer settled out. These crystals were filtered off, sucked to dryness, thoroughly washed with alcohol, and finally dried in a desiccator.

(3) *Reaction in liquid hydrogen sulphide.* Pure dry liquid hydrogen sulphide was prepared by the method of Antony and Magri [2] as modified by Quam [3].

RESULTS

Be—Mo—S. The product obtained after heating a mixture of the elements in proportion for $MoBe_2S_4$ for $6\frac{1}{2}$ hours at 800° had a greyish brown glittering appearance, and gave an X-ray pattern in which a number of MoS_2 lines (Bell and Herfert [4]) appeared. The remainder of the lines did not agree with the pattern for BeS given by Staritzky [5]. To check the correctness of this pattern a mixture of the elements in proportion for BeS was made up and heated in an evacuated tube for $6\frac{1}{2}$ hours at 800°. The X-ray pattern of the whitish product agreed with that of BeS [5]. It was concluded that the remainder of the lines in the pattern obtained for the Be—Mo—S system were not due to BeS, and it was observed that they showed a regular increase in $\sin^2\theta$ values.

The original powdered product was reheated at 800° for another 12 hours. The X-ray pattern then showed two definite MoS_2 lines and one other line of very weak intensity which did not correspond to a cubic face-centred structure, but the re-

mainder could all be indexed for a spinel $MoBe_2S_4$ with $a = 10.81$Å. The density found experimentally was 2·68 g.cm^{-3}., and the calculated value was 2·53 g.cm^{-3}.

The X-ray pattern (Cu radiation, nickel filter) is given in Table I.

TABLE I

Index	Estimated Intensity	Spacing d (Å)	Sin $^2\theta$ Expt.	Sin $^2\theta$ Calc.	d (Å) MoS_2	Calculated Intensities	
						Normal Spinel	Inverse Spinel
111	7	6·36	0·0147	0·0152		6·2	2·9
311	10	3·162	0·0593	0·0559		8·5	5·6
400	3	2·736	0·0793	0·0813		0·5	10·0
	1	2·301	0·1120				
440	10	1·912	0·1620	0·1626		10·0	7·2
622	9	1·631	0·2226	0·2235		2·9	< 0·1
	4	1·578	0·2386		1·586		
	5	1·532	0·2523		1·534		
800	2	1·352	0·3241	0·3251		1·6	0·5
662	3	1·238	0·3866	0·3861		3·6	2·1
844	8	1·102	0·4887	0·4877			
666	6	1·039	0·5497	0·5486			
880	5	0·953	0·6528	0·6502			
10,62	7	0·913	0·7121	0·7112			
12,40	6	0·854	0·8126	0·8128			
10,66	2	0·825	0·8727	0·8738			
995	1	0·790	0·9498	0·9499			
888	1	0·780	0·9744	0·9754			

Other systems containing Mo. The results of other attempts at preparing double metal sulphides by heating in evacuated tubes at 800° are summarised in Table II. Products were identified mainly with the aid of the A.S.T.M. Index[6]. Where MoS_2 was formed, the pattern given by Bell and Herfert[4] for synthetic rhombohedral MoS_2 usually gave better agreement than the hexagonal patterns of the natural products given in the A.S.T.M. Index.

TABLE II

Attempted Compound	Period of Heating (Hours)	Products Identified
$MoCu_2S_4$	24	MoS_2, CuS.
$MoHg_2S_4$	12	MoS_2, HgS.
$MoMn_2S_4$	12	MoS_2, MnS.
$MoPb_2S_4$	48	MoS_2, PbS.
$MoFe_2S_4$	12	MoS_2, pyrrhotite (magnetic)
$MoCa_2S_4$	12	Binary sulphides
$MoZn_2S_4$	12	Binary Sulphides
$MoBa_2S_4$	12	Binary sulphides
$MoCd_2S_4$	12	Binary sulphides
$MoNi_2S_4$	12	Binary sulphides

Precipitation from solution. The following double metal sulphides containing molybdenum were formed by precipitation from aqueous solution. The black precipitate of $MoAg_2S_4$ described by Berzelius[7] was obtained on addition of a silver nitrate solution to one of $(NH_4)_2MoS_4$. A brownish black precipitate of $MoHg_2S_4$, also described by Berzelius, was obtained by adding a solution of mercurous nitrate

to one of $(NH_4)_2MoS_4$. A brick red precipitate of $MoTl_2S_4$, not previously described, was formed when a solution of $TlNO_3$ was added to $(NH_4)_2MoS_4$ solution. This precipitate was thoroughly washed with hot water and then dried in a desiccator. Chemical analysis gave:—Mo 15·16%, theoretical 15·14%; Tl 64·58%, theoretical 64·60%; S 20·23%, theoretical 20·27%. A regular increment in the $\sin^2\theta$ values in the X-ray pattern of the product could be explained if a simple cubic structure, $a=11\cdot55$Å, is assumed for $MoTl_2S_4$. The X-ray pattern (Cr radiation, no filter) is given in Table III.

TABLE III

Index	Estimated Intensity	Spacing d (Å)	$\sin^2\theta$ Expt.	$\sin^2\theta$ Calc.
220β	1		0·0683	0·0687
220	10	4·042	0·0802	0·0824
311β	4		0·0965	0·0944
311	6	3·412	0·1125	0·1133
222	5	3·246	0·1244	0·1236
321	9	3·009	0·1446	0·1442
410	8	2·759	0·1720	0·1751
411	7	2·677	0·1826	0·1854
420	2	2·499	0·2096	0·2060
421	2	2·485	0·2168	0·2163
500	8	2·257	0·2569	0·2575
521β				0·2575
510	1	2·121	0·2676	0·2678
521	5	2·067	0·3062	0·3090
440	3	1·985	0·3323	0·3296
620	6	1·782	0·4124	0·4120
444β				0·4120
541	2	1·736	0·4344	0·4326
622	4	1·699	0·4536	0·4532
444	4	1·628	0·4945	0·4944
551	7	1·582	0·5237	0·5253
730	6	1·480	0·5976	0·5974

A new compound, $MoCu_2S_4$, was formed in a reaction between solutions of $(NH_4)_2MoS_4$ and Cu_2Cl_2, but the black precipitate was so finely divided that it did not give a clearly defined X-ray pattern. In an attempt to obtain larger particles the product was sintered in an evacuated tube at 300° for 5 hours. When it was X-rayed again it was found to have decomposed, and the X-ray pattern was that of MoS_2 and CuS. $MoCu_2S_4$ was then reprecipitated under more ideal conditions. Both solutions were kept at low concentration and the precipitation was carried out slowly in hot solution. The mother liquor was digested overnight on a waterbath to aid crystal growth. The precipitate was filtered off, washed and dried. A quantity was heated for a few hours at 100°. This gave a better X-ray pattern, but there were still only a few diffuse lines. These were in reasonable agreement with values for a spinel with $a=10\cdot6$Å. The density found experimentally was 3·73 g.cm.$^{-3}$, and the calculated value was 3.90 g.cm.$^{-3}$. Chemical analysis gave:—Mo 27·30%, theoretical 27·21%; Cu 36·00%, theoretical 36·17%; S 36·50%, theoretical 36·52%. The X-ray pattern is given in Table IV (Cu target, Ni filter).

Ta—Ti—S. A mixture of the elements in proportion for $TaTi_2S_4$ was heated for 6 hours at 800°. The product was a brittle golden material. The X-ray pattern was that of a cubic compound. A few weak lines, attributed to TiS and TiS_2, disappeared after reheating of the powdered product for a further 24 hours. The pattern for $TaTi_2S_4$ could best be explained on the basis of a body-centred cubic cell with $a=13\cdot22$. This is given in Table V (Mo radiation, Zr filter).

TABLE IV

Index	Measured Intensity	Spacing d (Å)	Sin $^2\theta$ Expt.	Sin $^2\theta$ Calc.	Calculated Intensities	
					Normal Spinel	Inverse Spinel
111	87	5·467	0·0199	0·0162	0·8	11
220	11	3·767	0·0418	0·0432	43	17
311	63	3·196	0·0581	0·0594	100	98
331	32	2·383	0·1044	0·1026	0·5	4
440	100	1·871	0·1693	0·1728	93	100

TABLE V

Index	Estimated Intensity	Spacing d (Å)	Sin $^2\theta$ Expt.	Sin $^2\theta$ Calc.
420	1	2·996	0·0140	0·0143
422	1	2·682	0·0175	0·0171
440	10	2·415	0·0216	0·0228
600	6	2·195	0·0261	0·0257
800	8	1·665	0·0454	0·0456
844	9	1·353	0·0687	0·0684
880	5	1·172	0·0914	0·0912
12,40	7	1.052	0·1136	0·1140
888	2	0·961	0·1361	0·1368
12, 10, 0	7	0·886	0·1596	0·1596
16,00	2	0·829	0·1826	0·1824
12, 12, 0	5	0·781	0·2061	0·2052
16, 80	3	0·740	0·2291	0·2280
12, 12, 8	3	0·707	0·2515	0·2508
16,88	2	0·676	0·2746	0·2736
20,40	3	0·651	0·2966	0·2964
16, 12, 10	1	0·606	0·3422	0·3420

Other systems containing Ta. Attempts were made to prepare compounds with the general formula TaM_2S_4, where M=Ba, Be, Ca, Cd, Co, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Zn. Heating the constituents for periods from 12 to 24 hours in evacuated tubes at a temperature of 800° resulted in each case in a mixture of binary sulphides.

Reaction in liquid hydrogen sulphide. Jander[8] has shown that solvolysis in liquid H_2S can produce metal sulphides. Attempts were made to produce double metal sulphides by bringing together two metal chlorides in liquid H_2S, but without success, as only binary sulphides were formed. The same applies to mixtures of metal acetates complexed with diethylamine.

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THE SPECTROCHEMICAL ANALYSIS OF CERTAIN MACRO- AND MINOR ELEMENTS IN PLANT ASH USING A CONSTANT CURRENT GENERATOR

by

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OPSOMMING

'n Metode om plantmateriaal, met behulp van 'n konstantegelykstroomboog as lig bron, te ontleed, word beskrywe. Die afhanklikheid van die resultate van die elektrode parameters word aangetoon. Die ontledingsresultate van 'n groep toetsmonsters in hierdie laboratorium verkry en die van drie ander laboratoriums vir die elemente kalium, magnesium, natrium, kalsium, barium, strontium, mangaan, yster, aluminium en koper word ook gegee en bespreek.

SUMMARY

A method for analysing plant materials for certain macro- and trace elements using a constant d.c. arc as light source is described. The dependence of the results on the electrode parameters is shown. The analytical results of a group of test samples obtained in this laboratory and in three other laboratories are given and discussed for the elements potassium, magnesium, sodium, calcium, barium, strontium, manganese, iron, aluminium and copper.

INTRODUCTION

When analysing plant materials spectrochemically the possible influence of some extraneous elements must always be considered. To minimise the effects of these extraneous elements it has become the practice, before exciting the samples, to dilute the ashed plant samples with a buffer. Aluminium oxide, [1] potassium sulphate, [2] sodium chloride, [3] germanium dioxide, [4] lithium carbonate, [5] etc., may be cited as examples. In this Laboratory lithium carbonate is generally used as the buffer material when analysing plant materials. For the excitation of the plant materials a d.c. arc is generally employed. The d.c. supply can be obtained from various sources of which the most common are batteries, generators or simple rectifier systems. The operation of the d.c. arc can, however, be considerably improved if the exciting e.m.f. of the analytical samples is, instead of batteries, etc., a constant direct current arc source [6], [7, 8, 9, 10]. The short period fluctuations as well as the general current change during the burning of plant ash samples, incorporated in a lithium carbonate base, in a normal d.c. arc, are practically completely resolved when a constant current generator is used. Current patterns of a 240 volt d.c. arc as obtained from a set of batteries, a mercury rectifier (3 phase input) and a constant current generator are shown in Fig. 1 (a), (b) and (c) respectively.

From Fig. 1 it can be seen that the general variation of current with time is approximately the same when using the batteries or the mercury rectifier as excitation source, but is considerably lessened when using a constant current generator.

The question arises, however, whether this improved operation of the constant current arc is generally translated to the analytical results. After a few trial exposures it became apparent that the operation of this direct current unit [10] is dependent on the parameters of the electrode system. It was accordingly decided to investigate the relative merits of the normal d.c. arc and constant current arc light sources when

used to excite plant and biological materials incorporated in a lithium carbonate base.

Results reported in this paper also include a comparison of analytical results for the elements potassium, magnesium, sodium, calcium, barium, strontium, manganese, iron, aluminium and copper, obtained in this Laboratory with those of three other laboratories.

COMPARISON BETWEEN NORMAL AND CONSTANT CURRENT ARCS.

(1) *Electrode forms used:*

As the performance of the constant current arc is dependent on the parameters of the electrode system, the tests with the different source units include some or all of the different top and bottom electrodes illustrated in Fig. 2.

(2) *Standard analytical conditions and photometric procedures:*

The standard analytical conditions and photometric procedures are listed below:

Spectrographs: (i) Jarrell-Ash 71·15 grating. Centre of plateholder set at 3,800 Å.

              (ii) Large Hilger prism. Camera set for the wavelength region 2,750 —4,600 Å.

Excitation   (a) 220 V batteries—13 amps. short circuited.

  sources: (b) 230 V constant current generator—7 and 10 amps.

            (c) 230 V mercury rectifier—13 amps. short circuited.

    (The central 4 mm. portion of the 9 mm. anode arc is photographed).

Sample Composition: See appendix.

Exposure: Samples burnt to completion, 2½-3 mins.

Plate type: (a) Kodak Uniform Gamma (for arc studies).

            (b) Kodak Spectrum Analysis No. 2 (for analysis curves).
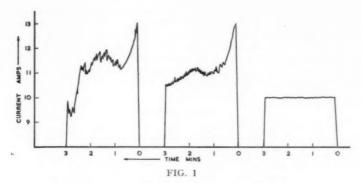
The recording conditions and the photometric procedures are those generally used in this laboratory[11], [12].

(3) *Method for evaluating the performance of the different light sources:*

Twenty sample holding electrodes belonging to each combination as listed in Tables 2 and 4 were filled with synthetic plant material, standard No. 3 (see appendix). These twenty filled electrodes were then divided into two equal groups. One group was excited using the constant current generator, while the other group was excited using the set of batteries or the mercury rectifier as the excitation source. Each group of ten exposures just filled a plate. After development of the plates the blackening of the lines listed in Table 1 was measured, the relative intensity ratios of the individual line pairs calculated, the average value determined and the percentage standard deviation calculated*. These results are given in Tables 2, 3 and 4.
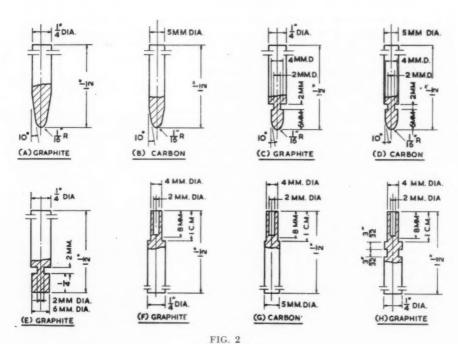
To make the comparison between the normal arc and constant current arc as objective as possible the two sets of exposures, using one type of combination of electrodes with different energy sources, were photographed one immediately following on the other.

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*The formulae used were $S = \left(\dfrac{\sum d^2}{n-1} \right)^{\frac{1}{2}}$ and $R = \dfrac{S}{x} \cdot 100\%$ where S is standard deviation; R, percentage standard deviation; d, difference between each value and the mean value; n, number of observation; x, mean value.

FIG. 1

Variations of current with time of a D.C. arc using 3 E.M.F. sources.
    (a) Batteries.
    (b) Mercury Rectifier.
    (c) Constant Current Generator.

FIG. 2

Electrode Forms used.

During the course of this investigation it was observed that the humidity of the air affected the burning of the test samples. Preheating of the sample for an hour at approximately 65°C before packing it into the electrodes and then keeping the sample filled electrodes hot on a thermostat controlled hotplate until burning, was found to improve the burn. Arc parameter combinations 4 and 6 of Table 4 were repeated as combinations 5 and 8 respectively using a sample dried in this manner and it can be seen from Table 4 that there is an apparent improvement in the results.

TABLE 1

Analytical line pairs

Element		Line pair
Barium	Ba	4554/Cr 4254
Strontium	Sr	4078/Cr 4254
Potassium	K	4044/Rb 4202
Magnesium	Mg	3336/Sn 3262
Sodium	Na	3302/Sn 3262
Copper	Cu	3274/Sn 3262
Calcium	Ca	3179/Sn 3262
Aluminium	Al	3082/Sn 3262
Iron	Fe	3021/Sn 3262
Manganese	Mn	2801/Sn 2840
	Mn	4030/Cr 4254

From the results given in Tables 2, 3 and 4 the following may be concluded:—

(a) The electrode parameters affect the reproducibility of the intensity ratios.

(b) By applying the "sign test"[13] to the results in Table 4, combinations 6, 8, 9 and 12 are the best, it being impossible to distinguish between these four combinations. The most satisfactory arc conditions are therefore given when

(i) the light source is a constant current arc unit,

(ii) the upper, purified carbon electrode has a hemispherical end, undercut to isolate it from the shaft of the electrode,

(iii) the lower sample carrying electrode is a high purity graphite rod, 0·25 ins. in diameter, 1·5 ins. long and with the sample carrying cavity having the dimensions 8 mm. long, 2 mm. diameter and a wall thickness of 1 mm.

(c) The higher arc current seems to improve the results.

(d) As a general conclusion it can be said that, if the samples are pre-heated to diminish the effect of air humidity, the constant current generator gives improved results over the batteries and the mercury rectifier.

THE ANALYSIS OF THE STANDARD PLANT SAMPLES.

(1) *Choice of plate types:*

The Kodak Uniform Gamma plate is basically the most suitable plate for analysis of the elements listed in Table 1, as these elements all have their analysis lines in the wavelength region 2,800 A-4,500 Å. The sensitivity of this type of plate was found, however, to vary considerably from batch to batch and to such an extent that each new consignment of plates necessitated the establishment of new analytical conditions. Replacing this type of plate by Kodak SpectrumAnalysis No. 2 plates (S.A. 2 plates) was accordingly considered. The gamma of the S.A. 2 plates is less consistent with wavelength than that of the Uniform Gamma type and they also have a much coarser grain. Care must also be exercised when processing these plates as they have a thicker gelatine layer than most other types of spectrographic plates normally used in a spectographic laboratory.

TABLE 2

Percentage relative deviations of the intensity ratios of the line pairs

7 amps arc current with constant current generator.
9 amps short circuited with batteries.
Spectrographic plate type: Kodak Uniform Gamma.
Jarrell-Ash Spectrograph.

Source*	Electrodes**		Mg 3336 / Sn 3262	Cu 3274 / Sn 3262	Na 3302 / Sn 3262	Ca 3179 / Sn 3262	Al 3082 / Sn 3262	Fe 3021 / Sn 3262	Mn 2802 / Sn 2839	Average
	Cath.	An.								
C.C.G.	E	F	17	10	13	23	13	12	10	14
Batt.	E	F	19	14	17	20	18	18	21	18
C.C.G.	B	F	9	15	12	15	14	9	9	12
Batt.	B	F	12	18	14	15	15	10	13	14
C.C.G.	D	F	7	4	8	7	7	7	11	7
Batt.	D	F	10	15	11	11	14	14	8	12

* C.C.G. Constant Current Generator.
   Batt. Batteries.
** Cath. Cathode.
    An. Anode.

TABLE 4

Percentage relative deviations using 10 amps arc current (13 amps short circuited with the batteries)
Spectrographic plate type: Kodak Uniform Gamma.

Combination	Source*	Electrodes		Ba4554 / Cr4254	Sr4078 / Cr4254	Mn4030 / Cr4254	Mg3336 / Sn3262	Na3302 / Sn3262	Cu3274 / Sn3262	Ca3179 / Sn3262	Al3082 / Sn3262	Fe3021 / Sn3262	Mn2802 / Sn2839	Remarks
		Cathode	Anode											
1	C.C.G.	B	F	21	16	12	7	12	10	14	11	11	10	
2	Batt.	B	F	19	19	13	10	21	13	25	17	11	21	
3	C.C.G.	A	F	27	18	10	9	25	5	21	21	25	12	
4	Batt.	A	F	17	14	14	11	15	18	14	15	18	12	Sample dried
5	Batt.	D	F	11	12	12	5	9	14	6	7	7	16	
6	C.C.G.	D	F	12	12	6	3	8	4	7	11	5	7	
7	Batt.	D	F	18	18	16	11	14	10	16	21	4	9	
8	C.C.G.	D	F	14	9	14	6	8	10	7	11	10	9	} Sample dried
9	C.C.G.	D	F	9	6	12	4	11	7	10	4	6	10	
10	C.C.G.	C	F	19	15	12	10	11	9	10	6	8	10	
11	Batt.	C	F	19	16	18	12	20	16	18	19	12	18	
12	C.C.G.	D	G	8	10	7	4	11	12	8	8	4	9	} Sample dried
13	C.C.G.	D	H	18	17	15	11	19	8	11	8	12	14	

*C.C.G. Constant current generator.
  Batt. Batteries.

TABLE 3

Percentage relative deviations comparing the constant current generator and . the mercury rectifier

Spectrographic plate type: Kodak S.A. 2.
Large Hilger spectograph.
Cathode D and Anode F Fig. 2.
10 A current with the constant current generator.
13 A current, short circuited, with the mercury rectifier.

Line pairs	Constant current generator	Mercury rectifier
Ba 4554/Cr 4254	7	12
Sr 4078/Cr 4254	12	17
K 4044/Rb 4202	8	10
Mg 3336/Sn 3262	9	11
Na 3302/Sn 3262	13	17
Cu 3274/Sn 3262	9	10
Ca 3179/Sn 3262	7	9
Al 3082/Sn 3262	8	9
Fe 3021/Sn 3262	11	16
Mn 2802/Sn 2839	5	11

To evaluate the performance of the S.A. 2 plates, a series of exposures was made on S.A. 2 plates using combination 8 (or 9) of Table 4. The standard and percentage standard deviations of the intensity ratios were calculated for the same group of elements given in Table 4. The percentage standard deviations of these line pairs, together with some results recorded in Tables 3 and 4 for ease of comparison, are given in Table 5.

TABLE 5

Percentage standard deviations on uniform gamma and S.A. 2 plates

Electrode combination: Cathode D, Anode F, Fig. 2.
Light source: Constant current generator.

Plate type Spectrograph	1† Uniform Gamma JA*	2†† J.A.*	3††† S.A. 2 L.H.**	4 J.A.*
Ba 4554/Cr 4254	14	9	7	6
Sr 4078/Cr 4254	9	6	12	10
Mn 4030/Cr 4254	14	12	8	5
Mg3336/Sn3262	6	4	9	5
Na 3302/Sn3262	8	11	13	9
Cu 3274/Sn3262	10	7	9	6
Ca 3179/Sn3262	7	10	7	7
Al 3082/Sn3262	11	4	8	4
Fe 3021/Sn3262	10	6	11	7
Mn 2802/Sn 2839	9	10	5	10

  * J.A.: Jarrell-Ash Spectrograph.
 ** L.H.: Large Hilger Spectrograph.
  † Comb. 8 (Table 4).
 †† Comb. 9 (Table 4).
††† Table 3.

Comparing either column 1 or 2 with column 4 in Table 5, it may be concluded that by applying the "sign test" the S.A. 2 and Uniform Gamma Plates are comparable for recording the spectra of the analytical elements listed in Table 5.

Comparison of the results given in columns 3 and 4 by means of the "sign test" shows the results in column 4 to be significantly superior to those in column 3. This means that the Large Hilger instrument seems to give results significantly inferior

to those obtained using the Jarrell-Ash spectograph. This is probably due to the curved spectral lines photographed on the prism instrument and strengthens the argument for having prism instruments fitted with curved slits in order to combat the coma effect.

FIG. 3

Analysis Curves of the Macro-Elements.

FIG. 4

Analysis Curves of the Trace Elements.

TABLE 6

Analytical values of the elements

Comparison of different spetrochemical procedures and a chemical analysis.

Laboratory	Cu p.p.m.	Mn p.p.m.	Fe p.p.m.	Al p.p.m.	Sr p.p.m.	Ba p.p.m.	Mg %	Na %	Ca %	K %	Sample
N.P.R.L.	8·7	46	165	262	108	366	0·88	0·02	2·39	2·72	4A Peach Leaves
O	5·5	58	172	285	115	212	0·79	0·02	2·34	2·28	
M	5·7	58	190	—	135	245	—	—	—	—	
C.S.	4	12	232	262	—	—	0·84	0·05	2·74	2·67	
F	—	—	—	—	—	—	—	0·059	2·66	2·63	
N.P.R.L.	13·8	41	303	500	230	76	1·18	0·02	2·25	1·68	4B Bean Leaves
O	9·6	42	368	615	152	45	1·12	0·01	1·94	1·4	
M	12·9	53	340	—	190	70	—	—	—	—	
C.S.	12	17	434	512	—	—	1·08	0·05	1·80	1·50	
F	—	—	—	—	—	—	—	0·05	1·84	1·61	
N.P.R.L.	13·9	162	263	462	74	153	2·21	2·73	1·47	3·47	4C Beet Leaves
O	8·4	179	252	457	78	177	2·38	3·15	1·35	2·82	
M	10·6	182	255	—	68	175	—	—	—	—	
C.S.	15	26	381	475	—	—	2·05	3·00	1·24	3·73	
N.P.R.L.	13·6	86	348	559	369	98	1·59	0·10	4·70	2·24	4D Gem Squash Leaves
O	9·5	107	391	703	279	92	1·36	0·02	3·94	2·08	
M	10·5	109	380	—	280	93	—	—	—	—	
C.S.	14	26	399	520	—	—	1·32	0·063	3·75	2·42	
F	—	—	—	—	—	—	—	0·062	3·79	2·47	
N.P.R.L.	6·8	129	154	325	32	29	0·41	0·01	1·04	1·29	4E Plum Tree Leaves
O	4·5	150	157	270	35	22	0·42	0·006	1·15	1·38	
M	5·8	141	150	—	42	34	—	—	—	—	
C.S.	9	60	239	325	—	—	0·40	0·055	1·20	1·24	
F.	—	—	—	—	—	—	—	0·044	1·33	1·32	

Sample	Lab										
4 F Lucerne	N.P.R.L.	15·0	25	334	608	39	31	0·41	0·35	1·27	1·76
	O	12·7	29	416	874	31	26	0·40	0·30	1·29	1·67
	M	19·5	29	310-460	—	31	28	—	—	—	—
	C.S.	15	10	383	612	—	—	0·44	0·33	1·00	1·40
4 H Tobacco (Healthy)	N.P.R.L.	8·8	68	259	531	68	56	1·59	0·02	2·31	1·47
	O	7·8	81	213	538	59	45	1·74	0·007	2·10	1·38
	M	9·3	62	210-310	—	38	41	—	—	—	—
	C.S.	7	33	325	531	—	—	1·50	0·045	1·63	1·45
4 I Tobacco (Sick)	N.P.R.L.	17·0	72	922	2500	27	25	1·46	0·03	2·05	2·09
	O	12·6	100	1246	2745	15	22	1·42	0·01	1·71	2·31
	M	17	50	1020	—	19	36	—	—	—	—
	C.S.	22	45	1199	2900	—	—	1·36	0·40	1·60	3·04
4 J Rumen content	N.P.R.L.	20·0	183	1013	5700	14	26	0·12	1·79	0·45	0·90
	O	21·7	177	960	4421	6	17	0·05	1·16	0·36	1·22
	M	22·3	67	940-1310	—	8	19	—	—	—	—
	C.S.	27	83	998	4700	—	—	0·04	2·13	0·34	1·00
1 O Citrus	N.P.R.L.	7·1	26	104	153	180	194	0·34	0·04	2·71	1·81
	O	6·1	28	94	181	189	328	0·27	0·02	2·34	1·67
	M	6·3	29	102	—	156	205	—	—	—	—
	C.S.	18	7	129	250	—	—	0·30	0·60	2·45	2·00

(2) *Analysis curves of the elements:*

As has been mentioned, when using the constant current generator, it was not possible to determine which of the electrode combinations, cathode D, anode F or cathode D, anode G, Fig. 2, gave the best results. The shaping of the carbon electrode is, however, more difficult than the shaping of the graphite electrode and it was decided to use the electrode combination cathode D, anode F with the constant current generator in order to establish the analysis curves of the elements.

(a) *Trace elements copper, aluminium, iron, manganese, barium and strontium.* To establish the analysis curves for these elements, the standards, prepared as described in the appendix, were photographed in triplicate, the individual exposures of each standard being photographed on a separate plate. The analysis curves of these elements are shown in Fig. 3.

For the elements, barium, copper, aluminium, iron and manganese, corrections for residual concentrations in the synthetic standards were made using the methods of Boettner[14] and Cholak and Storey[15]. Good agreement was found between the methods.

In order to determine whether these corrections really were valid, copper, aluminium, iron and manganese were separately added, in the form of solutions, in known amounts, to the buffer. The spectra of these mixtures were photographed and it was found that only at the correction value of each element, viz. 20 p.p.m. copper, 20 p.p.m. aluminium, 20 p.p.m. iron and 30 p.p.m. manganese, did the analysis lines of these elements become visible on the photographic plate. As a further control 20 p.p.m. and 40 p.p.m. of each element were added to two of the lower standards. These standards were then analysed and good recovery values were obtained.

The barium analysis curve required a residual contamination correction of 40 p.p.m. The strontium curve required no correction.

(b) *The macro-elements potassium, sodium, calcium and magnesium.* The analysis curves of the macro-elements required no correction and are illustrated in Fig. 4. The analysis line Mg 3336 is used in preference to the magnesium lines in the 2,800 Å region because these last named lines fall too near to the edge of the plate in a Littrow spectograph if barium and magnesium have to be determined simultaneously.

(3) *Comparison of analytical results with those from other laboratories:*

The analytical results of a series of standard samples, obtained using the method described above, are listed in Table 6. The results from three other laboratories are also listed and from these results the following can be concluded:—

Cu: The general agreement between the values obtained by this method and those given by Chemical Services seems to be fairly good. The agreement with those from the Macaulay Institute is also good. The Onderstepoort values seem generally to be slightly lower.

Mn: The spectrochemical results agree quite well with each other. The chemical results are generally low.

Fe: The general agreement in the results seems to be satisfactory. The chemical results are nearly always slightly higher than the others reported.

Al: Excepting for the results reported for Citrus, the agreement between the chemical results and the National Physical Research Laboratory results is very good.

Sr: The results of the three spectrochemical methods seem to agree satisfactorily.

Ba: Agreement between the results of the different laboratories is generally good.

Mg: The chemical and spectrochemical methods give comparable results.

Na: The results reported by the different laboratories are of the same order. The chemical results given for samples 41 and 10 seem to be erroneous. The flame photometric method is generally considered to be superior to d.c. arc methods. Results reported indicate that the d.c. arc method is less sensitive than the flame photometric method.

Ca: Results recorded for the constant current arc are generally higher than those obtained by other methods.

K: The results of all the methods recorded show satisfactory agreement.

CONCLUSION

Results given in this paper indicate that when a constant current d.c. arc unit is used the accuracy of d.c. excitation can be appreciably improved. This means that when this unit is used for routine analysis more accurate results can be obtained than when using normal d.c. excitation. Results given in Table 6 again stress the value of interlaboratory control analysis.

APPENDIX

Preparation of synthetic plant material standards:

For samples containing potassium, magnesium, sodium, calcium, silicon, strontium, barium, manganese, iron, aluminium, copper, lithium, chromium, tin and rubidium the method of preparation was similar to that described by Farmer. [2]

For establishing analytical curves for strontium, barium, manganese, iron, aluminium and copper, two mixtures were prepared with the following constituents:—

Mixture A: KH_2PO_4, 0.900g.; MgO, 0.250g.; Na_2CO_3, 0.45g.; SiO_2, 1.000g.; $CaCO_3$, 1.000g.; $SrCO_3$, 0.084g.; $BaCO_3$, 0.072g.; Mn_3O_4, 0.070g.; Fe_2O_3, 0.226g.; Al_2O_3, 0.299g.; CuO, 0.020g.; Li_2CO_3, 0.629g.; total weight 5.000g.

Mixture B: KH_2PO_4, 0.900g.; MgO, 0.250g.; Na_2CO_3, 0.450g.; SiO_2, 1.000g.; $CaCO_3$, 1.000g.; Li_2CO_3, 1.400g.; total weight 5.000g.

Eight standards were prepared by diluting mixture A with mixture B so that the concentration values in p.p.m. of the elements in the various standards were as follows:—

Ba, Sr, Mn: 10,000; 3,160; 1,000; 316; 178; 100; 56; 32.

Fe, Al: 31,600; 10,000; 3,160; 1,000; 562; 316; 178; 100.

Cu: 3,160; 1,000; 316; 100; 56; 32; 18; 10.

Standard 1 was undiluted mixture A.

Before burning in the arc the standards were mixed with buffer in the ratio 1:9.

A similar method of preparation was used for making up the macro-element standards. Two sets of standards were prepared, however, one set for Mg and Na the other set for Ca and K.

Mg-Na. Mixture A: SiO_2, 0·200g.; $CaCO_3$, 0·200g.; KH_2PO_4, 0·180g.; MgO, 0·166g.; Na_2CO_3, 0·230g.; Li_2CO_3, 0·024g.; total weight 1·000g.

Mixture B: SiO_2, 1·000g.; $CaCO_3$, 1·000g.; KH_2PO_4, 0·900g.; Li_2CO_3, 2·100g.; total weight 5·000g.

Ca-K. Mixture A: SiO_2, 0·200g.; $CaCO_3$, 0·250g.; KH_2PO_4, 0·348g.; MgO, 0·050g.; Na_2CO_3, 0·090g.; Li_2CO_3, 0·062g.; total weight 1·000g.

Mixture B: SiO_2, 1·000g.; MgO, 0·250g.; Na_2CO_3, 0·450g.; Li_2Co_3, 3·300g.; total weight 5·000 g.

The first standards were again the undiluted mixtures A and the other standards were obtained by diluting A with B in order to obtain the following concentrations of the macro-elements: 10·000%, 5·620%, 3·160%, 1·780%, 1·000%, 0·562%.

Before arcing the standards were again mixed with buffer in the ratio 1:9.

Ten grams of buffer was prepared by mixing together the following:

Li_2CO_3, 5·000g.; SP_2 grade spectrographic graphite powder, 5·000g.; RbCl, 0·283g.; 0·1% Cr solution, 1 ml.; 1·0% Sn solution, 1 ml.

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CHEMICAL INVESTIGATIONS OF PHOTOSENSITISATION DISEASES OF DOMESTIC ANIMALS
PART I. ISOLATION AND CHARACTERISATION OF STEROIDAL SAPOGENINS FROM TRIBULUS TERRESTRIS

by

W. T. DE KOCK and P. R. ENSLIN

OPSOMMING

Suurhidroliese van die ru saponiene isoleer uit *Tribulus terrestris*, 'n plant verantwoordelik vir hepatogene fotosensitiwiteit in skape, lewer diosgenien, ruscogenien, gitogenien en 25D-spirosta-3:5-dieen. Die afbou van ruscogenien na 1β,3β-dihidroksie-pregna-5:16-dieen-20-oon en 1β,3β-dihidroksie-pregn-5-een-20-oon word beskrywe.

SUMMARY

Acid hydrolysis of the crude saponins isolated from *Tribulus terrestris*, a plant responsible for hepatogenous photosensitisation in sheep, gave diosgenin, ruscogenin, gitogenin and 25D-spirosta-3:5-diene. The degradation of ruscogenin to 1β,3β-dihydroxypregna-5:16-dien-20-one and 1β,3β-dihydroxypregn-5-en-20-one is described.

INTRODUCTION

Tribulus terrestris L. (fam. *Zygophyllaceae*), an annual plant of the Karoo, is reported to be responsible for geeldikkop, a photosensitisation disease of sheep in South Africa. Quin and Rimington[1] showed the photodynamic agent to be phylloerythrin, a product of microbial chlorophyll digestion in the rumen of sheep. The accumulation of this fluorescent pigment in the peripheral blood circulation results from a derangement of the liver caused by toxins produced in *Tribulus terrestris* under certain climatic conditions. Since the excretion of bile pigments is also affected, a general jaundice accompanies the symptoms of acute photosensitisation. The evasive and labile character of the liver toxins formed in *Tribulus terrestris* has been responsible for the failure of numerous attempts to isolate them[1].

Three years ago this Laboratory and the Division of Veterinary Services of the Department of Agriculture, Onderstepoort, embarked on a collaborative programme of chemical and chemical pathological research on geeldikkop, which had again broken out extensively in the North Western Cape Province.

In a preliminary note[2] the isolation of saponins from *Tribulus terrestris* and their hydrolysis to diosgenin and a dihydroxy sapogenin of unknown structure, provisionally named sapogenin B, was reported. The present paper describes the isolation and identification of three sapogenins in addition to a dehydration product of diosgenin. The isolation and physiological properties of the saponins will be dealt with in a forthcoming paper.

Crude, amorphous saponins were extracted from dried *Tribulus terrestris* plants according to the method of Wall *et al*[3]. On hydrolysis with acid and chromatography on alumina, four crystalline substances were obtained *viz.* diosgenin, gitogenin, ruscogenin (sapogenin B) and 25D-spirosta-3:5-diene. The latter substance is probably an

artefact formed during acid hydrolysis of the saponins, a reaction recently studied by Peal[4]. The identity of these products was established by comparison with authentic specimens. The identity of sapogenin B with ruscogenin, first isolated from the rhizomes of *Ruscus aculeatus* L.,[5] was further proved by the preparation of various degradation products, *viz.* 1β,3β-dihydroxypregna-5:16-dien-20-one and 1β,3β-dihydroxypregn-5-en-20-one. The structure of ruscogenin has recently been established as 1β, 3β-dihydroxy-25D(*iso*)-spirost-5-ene.[6, 7, 8]

EXPERIMENTAL

All m.p.s. are corrected. Rotations were measured in chloroform solution. Ultraviolet absorption spectra were determined in 96% ethanol in a Unicam S.P.500 spectrophotometer. Infrared spectra were determined in a Perkin-Elmer Model 21 spectrophotometer. The spectra of the sapogenins were determined in chloroform and their acetates in carbon disulphide solution. Paper chromatograms were run according to a modification of a method described by McAleer and Kozlowski[9]. Whatman No. 540 filter paper was impregnated with a 1:1 mixture of benzyl alcohol and carbitol (diethylene glycol monomethylether) and the chromatograms were developed with cyclohexane. Spots were revealed by spraying with a solution of dimethylaminobenzaldehyde (0·25 g.) in ethanol (25 ml.), phosphoric acid (5 ml.) and perchloric acid (2-5 drops). On heating at 80° for 3 min. intense pink to brown-red spots were obtained.[10, 11]

Isolation of Sapogenins.—In general outline, the method of Wall *et al.*[3] was followed. Dried, ground *Tribulus terrestris* plants were exhaustively extracted with hot 96% ethanol. The concentrated extract was shaken with benzene, to remove chlorophyll and fatty impurities, and with butanol to remove saponins.

Evaporation of the butanol extract gave crude saponins as a light-brown foam. The yield varied between 1 and 2% in different batches. Hydrolysis of the crude saponins with 4N HCl and treatment of the product with methanolic KOH gave the crude sapogenins as a brown gum (yield 10-20%). Separation of the sapogenin mixture was effected by chromatography in benzene-chloroform solution (1:1) on alumina (ratio 1:30). Elution with benzene-chloroform (9:1, 2:3), chloroform, chloroform-ethanol (98:2) and ethanol gave four partly crystalline fractions.

25D-Spirosta-3:5-*diene.*—Benzene-chloroform (9:1) eluted *ca.* 2% of semi-crystalline material which was further purified by rechromatographing on alumina, crystallisation from methanol and sublimation at 140°/10^{-2}mm. to afford needles, m.p. 160-162°, [α]$_D$−195° (c, 0·8%), $\lambda_{max.}$ 235mμ, inflexions at 244 and 229mμ (log ϵ 4·30, 4·10 and 4·26 respectively)   [Found: C, 81·6, 81·7; H, 10·0, 10·3. Calc. for C$_{27}$H$_{40}$O$_2$: C, 81·8; H, 10·2%]. The infrared spectrum in chloroform solution showed strong bands at 865, 899, 920 and 982 cm.$^{-1}$ typical of the spiroketal side chain of a sapogenin[3]. The lack of a band in the 3400 cm.$^{-1}$ region indicated the absence of a free hydroxyl function. On hydrogenation over palladium on calcium carbonate in ethanol (containing 1% acetic acid) two moles of hydrogen were absorbed. The above diene was identified (m.p., mixed m.p. and infrared) as 25D-spirosta-3:5-diene by comparing it with an authentic sample prepared from diosgenin by fusion with potassium bisulphate in high vacuum. Recrystallisation from methanol and sublimation in high vacuum gave crystals, m.p. 157-158°.

Diosgenin.—Benzene-chloroform (2:3) eluted *ca.* 30% of partly crystalline material which was purified by rechromatographing on alumina and recrystallisation from acetic acid and methanol to afford needles, m.p. 204-205·5°, [α]$_D$−120° (c, 0·3%) [Found: C, 78·4; H, 10·3. Calc. for C$_{27}$H$_{42}$O$_3$: C, 78·2; H, 10·2%]. Acetylation (acetic

anhydride-pyridine) gave the monoacetate, m.p. 190-192°, $[a]_D -126°$ (c, 0·8%). This genin and its acetate were identified by comparison (m.p., mixed m.p., infrared spectra and paper chromatography) with an authentic sample of diosgenin isolated from *Dioscorea sylvatica* Ecklon.*

Ruscogenin.—Chloroform and chloroform-ethanol (98:2) eluted *ca.* 22% of semi-crystalline material which was purified by rechromatographing on alumina and crystallisation from acetic acid and methanol to afford needles, m.p. 213-215·5° (occasionally samples of constant m.p. 208-210° were obtained), $[a]_D -121°$ (c, 0·34%) [Found: C, 75·3, 75·3; H, 9·9, 9·8. Calc. for $C_{27}H_{42}O_4$: C, 75·3; H, 9·8%]. Acetylation (acetic anhydride-pyridine) gave a diacetate, m.p. 206°, $[a]_D -75°$ (c, 0·8%) [Found: C, 72·5, 72·6; H, 9·2, 9·2. Calc. for $C_{31}H_{46}O_6$: C, 72·3; H, 9·0%]. The lower melting genin gave an acetate with m.p. 190-194°. A comparison of the above genin and its acetate by means of m.p., mixed m.p., infrared spectra and paper chromatography with authentic samples of ruscogenin and its acetate†, proved their identity.

Gitogenin.—By washing the column with 96% alcohol, *ca.* 5% of a substance sparingly soluble in chloroform, was removed. Recrystallisation from acetic acid and from methanol afforded crystals, m.p. 263-267°. Acetylation (acetic anhydride-pyridine) gave the diacetate, m.p. 239-241°, $[a]_D -103°$ (c, 0·8%) [Found: C, 72·4; H, 9·7. Calc. for $C_{31}H_{48}O_6$: C, 72·1; H, 9·4%]. The m.p., mixed m.p. and infrared spectrum were identical with those of an authentic sample of gitogenin diacetate.‡ Alkaline hydrolysis of the acetate gave gitogenin, m.p. 271°, $[a]_D -69°$ (c, 0·8%) [Found: C, 75·1; H, 10·3. Calc. for $C_{27}H_{44}O_4$: C, 75·0; H, 10·3%].

1β,3β-dihydroxypregna-5;16-dien-20-one.—Ruscogenin diacetate (10·7g.), octanoic acid (14·2 ml.) (*cf.* Cameron *et al.*[12]) and acetic anhydride (5·4 ml.) were slowly heated to a temperature of 245° (acetic anhydride slowly distilled off) and kept at that temperature for 3 hr. The octanoic acid was subsequently removed by washing a benzene solution of the reaction product first with 2N-sodium hydroxide and then with water. Evaporation of the solvent afforded crude pseudoruscogenin acetate (brown syrup). This was dissolved in acetic acid (150 ml.), sodium acetate (2·5 g.) added and oxidised at 13° by the addition of a solution of chromic acid (4 g.) in 80% aqueous acetic acid (15 ml.) during the course of 15-20 min. After standing for 90 min. at 22°, excess methanol was added and the product worked up in the usual manner to give a brown oil. This was hydrolysed by shaking it for $3\frac{1}{2}$ hr. at 28° with a solution of aqueous potassium hydroxide (5 g.) in tertiary butanol (100 ml.) (*cf.* Wall *et al.*[13]). After dilution with water, the product was extracted with ether. Evaporation of the solvent and crystallisation from methanol gave plates, m.p. 223-232°; yield 3·6 g. Further purification was effected by filtration of the product in chloroform solution through a column of alumina, recrystallisation from methanol and acetone and sublimation in high vacuum at 160-180° to give plates, m.p. 234-240°, $[a]_D -37°$ (c, 0·8%), $\lambda_{max.}$ 238, 310-315 mμ (lon. ϵ 3·94 and 1·87 respectively). The $a\beta$-unsaturated ketone system at C_{20} appeared as typical bands at 1667 and 1740 cm.$^{-1}$ in the infrared spectrum (in chloroform solution) [Found: C, 76·2; H, 9·1. Calc. for $C_{21}H_{30}O_3$: C, 76·3; H, 9·2%].

1β,3β-dihydroxypregn-5-en-20-one.—The above 5:16-diene (408 mg.) was reduced over palladium on calcium carbonate catalyst (204 mg.) in ethanol. After 30 min. the reduction was interrupted (1·1 mole hydrogen absorbed) and the product crystallised from methanol to afford plates (194 mg.), m.p. 184-187°. Filtration of

this product in chloroform solution through alumina (20 g.) and crystallisation from methanol gave plates (88 mg.), m.p. 188-190°, $[a]_D + 38°$ (c, 0·5%) [Found: C, 75·9; H, 10·1. Calc. for $C_{21}H_{32}O_3$: C, 75·9; H, 9·7%]. A mixed m.p. with $1\beta,3\beta$-dihydroxypregn-5-en-20-one of m.p. 185-187°, prepared by H. Lapin from ruscogenin obtained from *Ruscus aculeatus* L., gave no depression. The two specimens had identical infrared spectra (in potassium bromide discs).

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